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SHORT COMMUNICATION

Effects of Thidiazuron, basal medium and light quality on adventitious shoot regeneration from *in vitro* cultured stem of *Populus alba×P. berolinensis*

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Abstract: The effect of Thidiazuron (TDZ), basal media and light quality on adventitious shoot regeneration from *in vitro* cultured stem of *Populus alba×P. berolinensis* were determined to establish a high efficiency shoot regeneration system from stem explants of *P. alba×P. berolinensis*. Stems of *Populus alba×P. berolinensis* were collected from cultured shoots *in vitro* derived from dormancy buds of 3-year-old seedlings. The stem explants were cultured on MS medium containing 0.02-mg·L^{-1} NAA (naphthaleneacetic acid), and 0.1, 0.3, 0.5 and 1.0 mg·L^{-1} concentrations of TDZ to determine the effect of TDZ on shoot regeneration. Three basal media, i.e. MS, woody plant medium (WPM) and B5, were used to test their influences of different media on adventitious shoot regeneration. Green, red, blue and yellow plastic films in comparison with florescent light as control were used to observe their effects on shoot regeneration. The results showed that different concentrations of TDZ had an evident influence on shoot regeneration. Lower concentration of TDZ (0.1 mg·L^{-1}) resulted in more adventitious shoot regeneration and higher concentration of TDZ (>0.1 mg·L^{-1}) inhibited shoot regeneration. Among different media, MS medium exhibited a high efficiency for shoot regeneration, followed by WPM medium, while B5 medium inhibited shoot regeneration. Normal light and yellow light exhibited better effects on shoot regeneration, compared with other light.

Keyword: *Populus alba×P. berolinensis*; stem explants; shoot regeneration

Introduction

P. alba×P. berolinensis, one of the new male hybrid tree species in China, grows fast and is cultivated in large area in northern China. It is used as an excellent resource for paper pulp and producing wood for solid wood products, including lumber and veneers. Conventional breeding program enabled us to select high-performance cultivars with regard to growth rate, adaptability, and yield in terms of wood and fiber quality or biotic and abiotic tolerance. However, improvement of trees by conventional breeding measure is constrained because tree species have a relatively long life span. In recent years, genetic transformation has offered an alternative in breeding and exhibited tremendous potential for tree improvement. Although, the biotechnological approach offers a potential for genetic improvement of seedlings of *P. alba×P. berolinensis*, the success of genetic manipulation depends largely on the availability of an efficient plant regeneration system. At present, studies on shoot regeneration of *populus* have been well reported (Noël et al. 2002; Dai et al. 2003; Thakur et al. 2005), but they are limited to very few genotypes and very few reports focused on shoot regeneration from stem explants of *P. alba×P. berolinensis*. Therefore, in the present study, we first established a high efficiency shoot regeneration system from stem explants of *P. alba×P. berolinensis*, which is the key step for genetic transformation of this tree species, and several factors influencing shoot regeneration, including Thidiazuron (TDZ) concentration, basal medium, as well as light quality was investigated. This article aims at revealing the effects of TDZ concentration, basal medium, as well as light quality on the production of adventitious shoots.

Materials and methods

Plant materials

Stems of *Populus alba×P. berolinensis* were collected from cultured shoots *in vitro* derived from dormancy buds of 3-year-old seedlings of *P. alba×P. berolinensis* in Maoershan Experimental Forest Farm of the Northeast Forestry University, Heilongjiang, China. *In vitro* shoots were maintained by sub-culturing at 4-week intervals on MS medium supplemented with 20 g·L^{-1} sucrose and 7 g·L^{-1} agar.

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Experimental design

To determine the effect of TDZ on shoot regeneration, the stem explants were cultured on MS medium containing 0.02-mg·L^{-1} NAA, and various concentrations of TDZ (Table 1), as well as 30-g·L^{-1} sucrose and 7-g·L^{-1}agar. After 4 weeks of culture, the frequency of the stem regeneration and the number of adventitious shoots per stem were recorded.

The influence of MS, woody plant medium (WPM) and B5 medium on shoot regeneration was investigated. The explants were cultured on the above three mentioned basal media containing 0.1-mg·L^{-1} TDZ, 0.02- mg·L^{-1} NAA, 30- g·L^{-1} sucrose and 7-g·L^{-1} agar. After 4 weeks of culture, the frequency of the stem regeneration and the number of adventitious shoots per leave were recorded.

Green, red, blue and yellow plastic films in comparison with florescent light as control were used to observe their effects on shoot regeneration. MS medium containing 0.1-mg·L^{-1} TDZ, 0.02-mg·L^{-1} NAA and 7-g·L^{-1} agar were used as basal medium. After 4 weeks of culture, data of stem regeneration frequency and adventitious shoots per stem were collected.

Culture conditions

All media were adjusted to pH 5.8 prior to autoclaving at 121°C for 20 min. Cultures were incubated at (25 ± 2) °C, under a 16-h photoperiod at photosynthetic photon flux of 40 μmol·m^{-2}·s^{-1} provided by white fluorescent lamps unless stated otherwise.

Statistical analysis

Each treatment consisted of 20 explants in triplicate. The number of adventitious shoots per explant was counted. The plant regeneration frequency (%) was calculated as the total number of explants producing shoots over the total number of explants inoculated. Means and Standard errors were used for statistical analysis.

Result and discussion

Effects of TDZ on shoot regeneration

TDZ，a synthetic phenylurea, has been reported to be the most active cytokinin-like substance for shoot induction in plant tissue culture (Huetteman et al. 1993; Murthy et al. 1998), and it is also effective in terms of shoot regeneration in many recalcitrant species (Pelah et al. 2002; Schween et al. 2002; Liu et al. 2003; Mithila et al. 2003).

In the present study, preliminary results showed that TDZ was superior over 6-benzyladenine (BA) and kinetin (KT) in promoting shoot regeneration from stem explants (data were not shown). Thus, TDZ was investigated in depth for its effect on shoot regeneration. We found that concentrations of TDZ affected shoot regeneration greatly. Lower concentration of TDZ was favored to shoot regeneration for *P. alba* ×*P. berolinensis*, which is in agreement with the similar result reported by Ledbetter and Preece (2004). The medium containing TDZ of 0.1mg·L^{-1} is the most efficiency and had 94% shoot regeneration frequency, with 4.6 shoots per explant. On this medium, the explants began to enlarge after one week culture and began to produce multiple shoot primordial (Fig.1, A) after two weeks of culture, which developed into adventitious shoots (Fig.1B) in the following two weeks. However, higher TDZ concentration inhibited shoot regeneration. As TDZ concentration increased from 0.1 mg·L^{-1} to 1.0 mg·L^{-1}, shoot regeneration frequency decreased from 94% to 23% accompanied with fewer adventitious shoot (Table 1). Moreover, on the medium containing TDZ (>0.1 mg·L^{-1}), the explants produced adventitious shoots more slowly than those on the medium containing TDZ of 0.1 mg·L^{-1}.

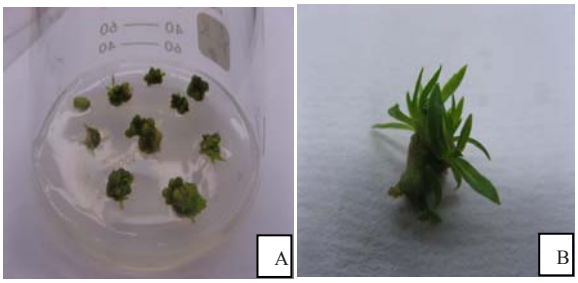

Fig. 1 The adventitious shoots from stem segment of *P. alba* ×*P. berolinensis* on shoot induction medium

A---- after two-weeks culture; B---- after four-weeks culture.

Table 1. Effects of TDZ on shoot regeneration from stem explants of *P. alba* ×*P. berolinensis*

TDZ (mg·L^{-1})	Explants producing shoots (%)	Number of shoots per explant
TDZ 0.1	94±0.89	4.6±0.45
TDZ 0.3	46±0.77	1.1±0.67
TDZ 0.5	30±0.18	0.7±0.38
TDZ 1.0	23±0.33	0.6±0.85

Effects of basal medium on shoot regeneration

As is shown in Table 2, MS medium was favored to shoot regeneration, followed by WPM medium, but B5 medium inhibited shoot regeneration. MS medium has been frequently used for shoot induction (Siril et al. 1996). On the B5 medium, the adventitious shoot occurred more slowly than on other media. It was also found that adventitious shoot grew slowly and exhibited in yellow color. These responses from different media in shoot regeneration might be caused by nutrition difference in media. The most prominent difference between MS, WPM and B5 media is the lower ratio of NH_4^+/NO_3^- in B5 medium, although other difference in the media cannot be excluded as contributing to this response.

Effects of light quality on shoot regeneration

The influence of green, blue, yellow and red light on regeneration ability was tested using normal light as control. The stem

explants were exposed to different light wavelengths during the first 30 days. Our experimental results clearly demonstrate that light quality has an evident influence on the morphogenesis of *P. alba*×*P. berolinensis*. The highest number of shoots was found under the normal light and yellow light, followed by blue films, while red and green film inhibited regeneration of adventitious shoots (Table 3). Under normal light or yellow light, the adventitious shoots grew faster than under other color films. While yellow light promoted shoot production, compared with other color films, and it had no effect on shoot number compared to the control under normal light.

Table 2. Effects of basal medium on shoot regeneration from Stem explants of *P. alba* ×*P. berolinensis*

Basal medium	Explants producing shoots (%)	Number of shoots per explant
WPM	93±0.35	3.2±0.85
B5	57±0.23	1.7±0.28
MS	100	5.1±0.32

Table 3. Effects of light quality on shoot regeneration from stem explants of *P. alba* ×*P. berolinensis*

Light quality	Explants producing shoots (%)	Number of shoots per explant
Red light	87±0.28	3.0±0.78
Blue light	83±0.34	4.7±0.37
Green light	67±0.25	2.9±0.32
Yellow light	93±0.33	5.6±0.54
Control	95±0.82	5.8±0.19

Light quality plays a vital role in plant development, morphogenesis, photosynthetic and metabolism. In our case, color films had significant effects on shoot regeneration. It may be due to the fact that the light quality affected the physiological and biochemical processes during adventitious shoot regeneration. Red and green films were not suitable for shoot regeneration due to lower ultraviolet radiation under red and green films (Qin et al. 2005). In other studies, however, it was reported that red and green light greatly promoted shoot regeneration (Burritt et al. 2003; Qin et al. 2005).

In conclusions, TDZ concentration, basal medium and light quality all had obviously effects on shoot regeneration. Under normal light or yellow light, the explants cultured on MS medium supplemented with 0.1-mg·L^{-1} TDZ and 0.02- mg·L^{-1} NAA produced regenerated shoots at high frequencies with more shoots per explant. This protocol could be useful for clonal propagation and genetic transformation for seedlings of *P. alba* ×*P. berolinensis*.

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